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A new mouse model for the neurodevelopmental ciliopathy Joubert syndrome

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Abstract

Recent recognition of the key role of primary cilia in orchestrating human development and of the dire consequences of their dysfunction on human health have placed this small organelle into the spotlight. While the causal link between mutations in ciliary genes and central nervous system malformations and dysfunction is well established, the mechanisms by which primary cilia dysfunction acts on development and function of the CNS remain partly unknown. The recent article by Bashford and Subramanian in the *Journal of Pathology* describes a new mouse model for the neurodevelopmental ciliopathy Joubert syndrome, supporting a role for ciliary-mediated Hedgehog signaling on proliferation, survival and differentiation of cerebellar granule cell progenitors.

Key words:

Ciliopathies, Joubert syndrome, Molar Tooth Sign, Cerebellar malformation, Mouse model, Talpid3

In a recent issue of the *Journal of Pathology*, Bashford and Subramanian [1] describe a new mouse model for the neurodevelopmental ciliopathy Joubert syndrome (JBTS). The relevance of studying this rare disorder lies in its link to primary cilia, small organelles whose key role in human development and disease has become obvious in recent years and is illustrated by the increasingly large group of disorders called ciliopathies.

Ciliopathies are Mendelian disorders defined by their shared underlying pathomechanism linked to dysfunctional primary cilia. These ubiquitous microtubule-based organelles have been compared to cellular antennae, since they play crucial functions in sensing, transducing and regulating environmental signals. Transmitted signals depend on the cell type and the developmental stage and include light sensation or olfaction, but also signaling pathways such as Hedgehog or Wnt (wingless) signaling during development and cell homeostasis [2]. Ciliopathies are also unified by a set of recurring overlapping phenotypes, among which central nervous system (CNS) anomalies are common. The role of cilia on developing and mature neurons and the precise pathomechanisms leading from dysfunctional primary cilia to CNS dysfunction and malformations are only partly understood to date. The recent manuscript by Bashford and Subramanian presents a detailed description of the CNS phenotype of a new conditional mutant in *KIAA0586/TALPID3*, one of the genes associated with JBTS.

JBTS is characterized by a highly specific cerebellar and brain stem malformation called the Molar Tooth Sign due to its appearance on axial brain MRI. This complex malformation comprises vermis hypoplasia or aplasia, long, thick superior cerebellar peduncles and a deep interpeduncular fossa [3]. In addition, patients with JBTS may display a number of additional CNS abnormalities including agenesis of the corpus callosum, encephalocele, hydrocephalus, posterior fossa cysts, cerebellar and cortical heterotopias, and polymicrogyria [4]. *KIAA0586/TALPID3* is one of the ~35 genes in which mutations cause this specific hindbrain malformation [5]; other JBTS genes for which mouse models have been described include *TCTN1*, *AHI1*, *CEP290, RPGRIP1L* or *TMEM67*.

Previous work has identified some hints as to the pathogenesis of the cerebellar malformation in JBTS. The study of human JBTS fetal samples, mostly harboring mutations in the *CEP290* gene, provided evidence for disrupted Sonic Hedghehog (SHH) signaling [6]. This has however not been unequivocally recapitulated in all mouse models of the disorder so far. Many constitutive mouse knock-outs in JBTS-associated genes lead to embryonic lethality, such that only embryonic stages were analyzed, which often showed disrupted HH signaling and aberrant neural tube patterning resulting in major CNS defects such as holoprosencephaly [7]. A few mouse models

for JBTS displaying cerebellar phenotypes have been described, including the *Ahi1* mouse reported by Lancaster et al, which had a smaller cerebellum compared to wildtype, decreased vermis size, mild foliation defects, decreased granule cell proliferation but no Hedgehog signaling defects [8]. This model showed in addition a mild midline fusion defect at embryonic stages with decreased Wnt signaling in the cerebellar midline. A *Cep290* mouse knockout reported in the same work had an even milder but similar phenotype. An *Rpgrip1l* knock-out mouse was reported to display an abnormal cerebellum but no detailed description of the phenotype is available [9]. Interestingly, this mutant also displays forebrain abnormalities related to aberrant Hedgehog signaling [10]. A *Tmem67* mouse knockout displayed either a cerebellar phenotype reminiscent of JBTS or a more severe CNS malformation recapitulating the related ciliopathy Meckel syndrome, thereby illustrating the phenotypic variability of this disorder group and supporting a role for genetic modifiers [11]. The suggested modifiers likely explain the difference in ciliogenesis defects observed between the two *Tmem67* mutant groups, as cilia were lacking in the Meckel-like mice but were still present, albeit abnormal, in the JBTS-like mice, despite the two mutant groups harboring the same *Tmem67* mutation. These differences in ciliary morphology were correlated with different consequences on Hedgehog and Wnt signaling pathways. Importantly, this paper nicely demonstrated a role for both signaling pathways underlying the CNS abnormalities. Finally, conditional knock-outs in two genes known to be required for cilium formation but not associated with JBTS in patients (so far?), *Ift88* and *Kif3a*, resulted in a smaller post-natal cerebellum and foliation abnormalities with decreased granule cell proliferation and failure of expansion of the neonatal granule cell progenitor pool [12].

The recent manuscript by Bashford and Subramanian presents a conditional *Talpid3* knock-out mouse with a cerebellar phenotype reminiscent of the human hindbrain malformation. *Talpid3* cKO mice display ataxia and a markedly hypoplastic cerebellum with a significantly smaller vermis, similar to findings in patients with JBTS. The authors present a very thorough description of the anomalies underlying these phenotypes, finding decreased granule cell proliferation, as previously reported in humans and in *Ift88/Kif3a* cKO mice. Moreover, increased apoptosis in the external granule cell layer is found to contribute to reduced granule cell density. Premature maturation, misorientation and arrested migration of granule cells from the external granule cell layer further contribute to the reduced number of granule cells in the inner granule cell layer. In addition, the authors describe abnormalities in the glial scaffold. Importantly, they observe disorganization of the Purkinje cell layer with mislocalized Purkinje cells (PC) in the inner granule cell and molecular layers. While the overall number of PCs is reduced in these smaller cerebella, they do show a relatively increased density in the context of a small cerebellum. Finally, abnormal

dendritic arborization of Purkinje cells and a disorganization of excitatory synapses formed by climbing fibers and granule cell parallel fibers shows that the cerebellar circuitry is affected. This study provides the most comprehensive description of a mouse model reminiscent of the human cerebellar phenotype of JBTS to date.

From a mechanistic point of view, the authors show that loss of Talpid3 results in loss of cilia on cerebellar neurons in the external and internal granule cell layers, which is consistent with previous findings in human patient fibroblasts [13], zebrafish photoreceptors [14] or in the neural tube of chick embryos [15]. Interestingly, this severe reduction in cilia numbers in *Talpid3* cKO mice does not result in the same severe malformation as described in the *Tmem67* Meckel-like mutants without cilia, but a phenotype that is closer to that of *Tmem67* Joubert-like mutants which still display cilia. So a simple correlation between severity of ciliogenesis defects and severity of CNS malformation does not appear to apply. Bashford and Subramanian further show an effect on Hedgehog signaling in their *Talpid3* cKO model, with decreased levels of Gli1, Ptch1, Gli2A but increased Gli3R. These findings, together with previous work showing the importance of primary cilia for Hedgehog signaling, and of Hedgehog signaling for cerebellar granule cell proliferation, support dysregulation of this pathway as the major mechanism causing the hypoplastic vermis seen in JBTS patients. Notch signaling appears to be unaffected in this model, and classical Wnt readouts are also normal, but the authors observe a decrease in Wnt7a, which will be interesting to follow-up upon.

The new JBTS mouse model described in this work now provides an excellent opportunity to investigate the role of primary cilia in development of the CNS and to understand the pathomechanisms underlying the Molar Tooth Sign. Conclusions from this work suggest that decreased granule cell proliferation coupled with abnormal migration and differentiation of GCPs as well as increased apoptosis appear to play a major role in the pathogenesis of the Molar Tooth Sign. However, many open questions remain. Are the observed anomalies of glial scaffold or Purkinje cells, including the defective circuit formation, a secondary defect of cerebellar development caused by aberrant granule cell behavior or a direct consequence of ciliary dysfunction? Another question pertains to the predominant effect of JBTS gene dysfunction on the cerebellar vermis; why are the hemispheres much less affected, if GCP proliferation is the main problem? While various studies provide evidence for involvement of both pathways, the relative roles of and interplay between Hedgehog and Wnt signaling also remain unresolved. Future work on the mouse model presented here will hopefully help address some of these questions.

Conflict of Interest Statement:

The author declares no conflict of interest.

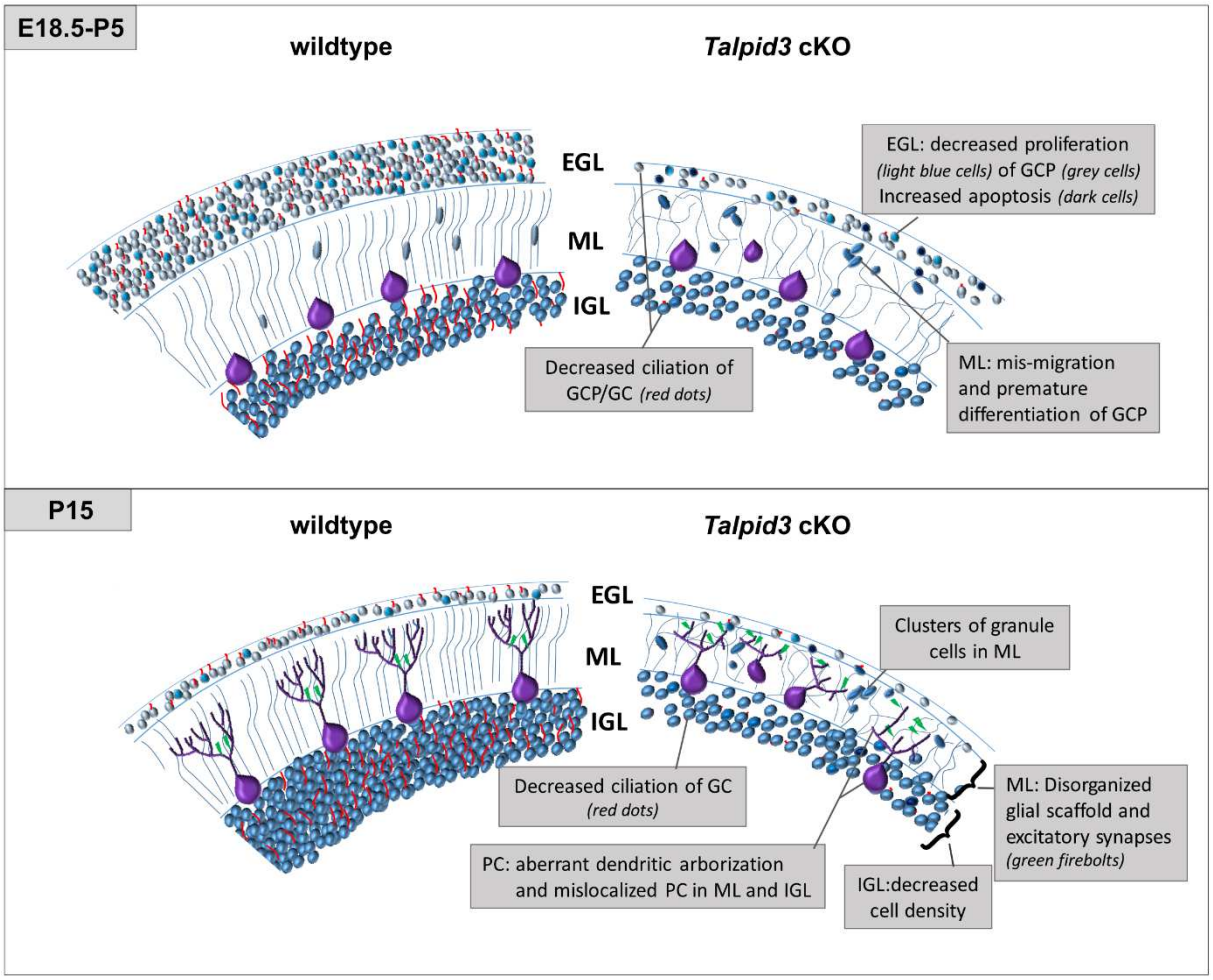

Figure 1: Abnormal cerebellar development in a new mouse model for JBTS.

Cerebellar findings in Talpid3 conditional knockout (cKO) mice compared to wildtype at early and later stages of cerebellar development: Bashford and Subramanian describe loss of cilia (red) on granule cell progenitors (GCPs) and granule cells (GCs) in the external granule cell layer (EGL) and the internal granule cell layer (IGL). At early stages, the EGL is thinner with decreased density of GCPs (grey cells), decreased proliferating GCPs (blue) and increased apoptotic GCPs (black). In the molecular layer (ML), abnormal migration of GCPs, which are tangentially oriented, is described as well as their premature differentiation. At later stages, the ML shows a disorganized glial scaffold and clusters of GCs. Purkinje cells (PC, magenta) are mislocalized in the ML and IGL and display abnormal dendritic trees with disorganized excitatory synapses (green firebolts). The IGL is thinner with decreased density of GCs.

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